

## BASIC SCIENCE

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# *Propionibacterium acnes*, Coagulase-Negative *Staphylococcus*, and the “Biofilm-like” Intervertebral Disc

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**Study Design.** Patients scheduled for spinal surgery were screened prospectively for a microbial presence associated with intervertebral disc specimens. Inclusion was limited to patients requiring surgery for any of five conditions: study patients with cervical spine intervertebral herniation (IVH), lumbar spine IVH, lumbar spine discogenic pain, and control patients with idiopathic scoliosis/Scheuermann’s kyphosis or trauma/neuromuscular deformity. Exclusion criteria included ongoing systemic infection, abnormal pre-operative white cell counts, documented or suspected spinal infection, or previous surgery to the involved disc.

**Objective.** The aim of this study was to test for an association between the presence of a bacterial entity in operated discs and a diagnosis of pathologic disc disease.

**Summary of Background Data.** An association has been described between microbial colonization and progressive intervertebral disc degeneration in 36 herniation patients undergoing microdiscectomies. A total of 19 patients had positive cultures on long-term incubation, with *Propionibacterium acnes* present in 84% of discs.

**Materials and Methods.** Discs were harvested during surgery, using strict sterile technique. Each disc was divided, with half the sample sealed in a sterile, commercially prepared anaerobic

culture transport container, and half fixed in formalin. Live specimens were cultured for bacteria at a university-affiliated laboratory in a blinded fashion. Fixed pathologic specimens were gram-stained and read by a board-certified pathologist.

**Results.** A total of 169 intervertebral discs from 87 patients were evaluated (46 males, 41 females). Positive cultures were noted in 76 of 169 discs (45%), with 34 discs positive for *P. acnes* and 30 discs positive for *Staphylococcus*. No pathologic evidence was seen of microorganisms, acute or chronic inflammation, or infection. Pooling the IVH and discogenic pain patients and contrasting them with control patients showed a significant association of IVH with positive bacterial cultures ( $\chi^2 = 15.37$ ;  $P = 0.000088$ ).

**Conclusion.** Endemic bacterial biofilms are significantly associated with IVH and discogenic pain.

**Key words:** biofilm, coagulase negative staphylococcus, disc herniation, intervertebral disc, low back pain, *Propionibacterium acnes*.

**Level of Evidence:** N/A

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Progressive spinal degenerative change, spinal deformities, and painful spinal conditions have been linked to the presence of bacterial biofilms. To evaluate a possible association between a microbial presence, progressive intervertebral disc degenerative changes, and symptomatic disc pathology, Stirling *et al.*,<sup>1</sup> in 2001, presented the first series of patients (36) whose discs were cultured after microdiscectomies for disc herniations. A 53% positive culture rate was noted on long-term incubation. The most prevalent bacterium was *Propionibacterium acnes* (*P. acnes*) in 84% of samples with coagulase-negative (CN) *Staphylococcus* in 11% of samples. However, this paper was extremely controversial.

Two recent systematic reviews concluded that disc infection may be linked to disc degeneration, but concluded that the available data were limited.<sup>2,3</sup> We seek to extend the available data using a rigorous prospective study design that was previously presented only in an abstract form.<sup>4</sup>

**TABLE 1. Bacterial Distribution Among Categories**

Bacterial Entities	Category I	Category II	Category III	Category IV	Category V
<i>P. acnes</i>	12	5	8	5	4
CN <i>Staphylococcus</i>	3	12	5	4	6

CN indicates coagulase negative; *P. acnes*, *Propionibacterium acnes*.

## MATERIALS AND METHODS

A prospective study was undertaken after obtaining the appropriate Institutional Review Board approval from the participating institutions. Informed consent was obtained from each patient after a careful explanation of risks and benefits.

Patients appropriate for inclusion were those who were already scheduled for surgical intervention through the established practices of five surgeons. All patients were in good health with normal white cell counts on complete blood cell (CBC) tests done pre-operatively. There were no age or ethnic exclusions. Patients were excluded from the study if they had ongoing systemic infection, previously documented or suspected infection of the spine, or previous surgery to the involved disc or discs.

Participation in this study did not modify in any way an individual patient's diagnostic work-up, surgical treatment, or postoperative care. Each surgeon was board certified in orthopedic surgery and spine fellowship trained. Prophylactic antibiotics were used as per routine. The patients understood that they would not be notified of their culture results, and even if they were culture positive, they would not be treated with additional antibiotic therapy, due to lack of specific guidelines.

Five diagnostic categories were specified with approximately 30 discs to be obtained in each of the first four groups and approximately 45 in the fifth group. The categories were as follows:

- I. Cervical spine intervertebral disc herniations;
- II. Lumbar spine intervertebral disc herniations;
- III. Lumbar spine discogenic pain (internal disc disruption syndrome);
- IV. Control-Idiopathic scoliosis/Scheuermann's kyphosis;
- V. Control-trauma and neuromuscular deformities.

The trauma patients in Group V were both acute and chronic at the time of their surgery for stabilization/reconstruction.

Discs in Groups I, III, IV, and V were harvested from an anterior approach to the spine. These involved complete discectomies with either an open or a laparoscopic/thoracoscopic procedure. Discs in Group II were obtained by posterior microdiscectomy technique.

Immediately upon removal, using strict sterile technique, each disc was divided into two portions. The first was immersed into a sterile, commercially prepared anaerobic culture transport medium container and sealed. The second

portion was placed in an individual formalin container. All labeling of the transport containers, as well as laboratory processing used a unique patient number, to preserve patient confidentiality and to maintain the study blind in the laboratory. A single university affiliated laboratory processed all cultures in a blinded fashion. All pathologic specimens were prepared, gram-stained, and read by a board-certified pathologist. The culturing process was comparable to that described by Stirling *et al.*<sup>1</sup>

A total of 169 intervertebral discs were obtained from 87 patients. There were 46 males and 41 females, with an average age of 40.0 years ( $\pm 15.2$ ). Pre-operative diagnostic and therapeutic spinal injection procedures were recorded for each patient with the concern that these could potentially be a source of bacterial contamination.

Due to the wide variety of spinal clinical pathology encompassed in this study, and the referral nature of the involved surgeons' practices, this resulted in a broad spectrum of abnormalities. To determine whether there was a correlation between magnetic resonance imaging (MRI) disc space changes and culture results, a board-certified neuroradiologist evaluated the Modic grade of the involved discs in a specific subset of patients whose studies were performed at two, high-quality referral facilities.<sup>5</sup> Medical insurance plans excluded some patients from having their pre-operative studies performed at the desired facilities. MRI scans were not indicated clinically in all of the patients.

## STATISTICAL ANALYSIS

Patient demographic and surgical episode data collected during the study were considered as potential predictors in the model. Demographic and surgical episode variables were compared between the culture-positive and culture-negative groups; a *t* test was utilized for numeric data and Fisher exact tests were utilized for categorical data.

## RESULTS

Positive cultures were noted in 76 (45%) of the 169 discs, while 93 discs, 55% showed no growth. Thirty-four discs were positive for *P. acnes* (20% of total discs/45% of positive cultured discs), with eight of these having an additional single organism growth. Thirty discs grew coagulase-negative (CN) *Staphylococcus* (18% of total discs/40% of positive cultured discs), with five cultures having a single additional organism growth. All of the *Staphylococcus* cultured in the study were CN. Of the remaining bacterial species cultured, each individually involved less than 4% of the total number of discs. The distribution of the

**TABLE 2. Amplified Fragment Length Polymorphism (AFLP) of *P. Acnes***

Pathological category	Electrophoresis Gel Pattern			
	IA	IB	II	III
Cervical disc	1	2	3	3
Lumbar disc		1	2	
Lumbar discogenic		1	1	2
Idiopathic deformity (Control)				2
Trauma (Control)	1			1

two predominant cultured bacterial entities, according to the five categories, is represented in Table 1.

There was no significant association with patient gender ( $P=0.0761$ ) or age ( $P=0.1086$ ). Individual surgeon's positive culture rates averaged 35% to 40%.

The 30 discs positive for CN *Staphylococcus*, were further subcultured, revealing 71% *Staphylococcus epidermidis*, 12% *Staphylococcus auricularis*, 12% *Staphylococcus laminiis*, and 5% other.

A subset of 20 of the original 34 *P. acnes* cultures were analyzed by using amplified fragment length polymorphism (AFLP). Those electrophoresis gel patterns (Table 2) were then arranged into the four types designated at the time: I, II, III, and IV. These would now be, in the order: IA, IB, II, and III, respectively.

Evaluation of specimens by a pathologist showed no evidence of micro-organisms, acute or chronic inflammation, or infection, following extensive light microscopic review of the fine-cut, gram-stained specimens of each disc.

The MRI scans of 41 discs (27 patients) were evaluated in a blinded fashion by a board-certified neuroradiologist. Assessment was subsequently made comparing their Modic grade, as determined by T<sub>1</sub> and T<sub>2</sub>-weighted imaging, to their culture status. There was no correlation between Modic grade disc category and culture results ( $P=0.4302$ ), suggesting that a bacterial presence does not necessarily lead to pathologic changes visible by MRI.

There was no statistically significant increase in the positive disc culture rate associated with any of the relevant pre-operative injection procedures ( $P=0.9488$ ).

## DISCUSSION

The concept of a human microbiome community within skeletal elements is an area of emerging interest. Similarly, there has been a growing focus on the effects of the gut microbiome and its variations on spondyloarthritis and arthritic conditions in general.<sup>6–16</sup> Previous misconceptions that particular regions of the human body were sterile are now being more realistically evaluated with molecular and DNA assessment techniques and proven otherwise.<sup>16</sup>

The two predominant bacteria cultured in this study have been shown to have a number of similarities. Both are non-motile, nonspore-forming, gram-positive, facultative anaerobics. *P. acnes*, a rod, normally inhabit the skin, oral cavity, and gastrointestinal tract. A minimum incubation duration of

14 days is recommended, due to its slow growth and ease of domination by other, more robust, bacteria.<sup>17</sup> A combination of nucleotide sequencing (tly and recA), as well as MultiLocus Sequence Typing (MLST) has confirmed the four highly distinct evolutionary types previously noted (IA, IB, II, and III); each varying in virulence and inflammatory response.<sup>17</sup> Skin acne is typically associated with type IA, possibly IB, while types II and III are found in endodontic lesions and prostate cancer specimens.<sup>18–20</sup> The *P. acnes* found on healthy skin does not form significant biofilms, whereas the more virulent forms do.<sup>21</sup> *P. acnes* have been cultured from a wide variety of orthopedic implants, lymph nodes of patients with sarcoidosis and bone biopsies, and synovial fluid of patients with the SAPHO (Synovitis-Acne-Pustulosis-Hyperostosis-Osteitis) syndrome.<sup>22–24</sup> Interestingly, in *P. acnes* infected spinal implants, patients are usually afebrile and biological markers of inflammation are rarely abnormal.<sup>25</sup> Because the bacteria can be quite difficult to culture from extracted implants, a technique involving vortexing and sonication to break down the biofilm and expose the bacteria has been recommended.<sup>26</sup> *P. acnes* can survive and persist inside of macrophages after phagocytosis, though it does not appear to replicate.<sup>27</sup> Similarly, polymorphonuclear cells (PMNs) and monocytes have been shown to be unable to degrade some types of *P. acnes in vitro*.<sup>27</sup>

*S. epidermidis* (the predominant *Staphylococcus* cultured) is a cocci that has a number of adhesions, allowing for optimal attachment to surfaces.<sup>28</sup> It can degrade elastin, fibrinogen, fibronectin, survive in macrophages, and avoid phagocytosis via its biofilm.<sup>28</sup>

It is estimated that no more than 1% to 2% of microbial species have been cultured; evolving molecular techniques are now providing a window to this new world.<sup>1</sup> The results of this study appear to be reflective of bacterial micro-communities protected in a biofilm, both of their own creation and due to the environment naturally afforded by the intervertebral disc itself. That is, by being avascular, well hydrated, low in oxygen tension, and having a neutral pH, a disc is already “biofilm-like.”<sup>6</sup> The positive culture rate of 45% overall could be consistent with difficulty in separating bacteria from their biofilm, as well as trying to culture the phylogenetically modified bacterial version that rapidly evolves, once encased in a biofilm. When these specimens were cultured, no special “separation” techniques were utilized to remove the bacteria from their biofilms or the disc itself.

**TABLE 3. Discs After Division Into Categories**

Category	I. Cervical	II. Lumbar	III. Lumbar Discogenic	IV. Deformity (Control)	V. Trauma (Control)
Total discs (169)	30	31	31	33	44
% Positive culture (n)	63% (19)	65% (20)	48% (15)	30% (10)	27% (12)
<i>P. acnes</i>	63% (12/19)	25% (5/20)	53% (8/15)	50% (5/10)	33% (4/12)
CN <i>Staphylococcus</i>	16% (3/19)	60% (12/20)	33% (5/15)	40% (4/10)	50% (6/12)

CN, coagulase negative; *P. acnes*, *Propionibacterium acnes*.

That the light microscopic evaluation of the disc specimens, having been Gram stained, showed no evidence of acute or chronic inflammatory change, or bacteria, would be in keeping with a “biofilm within a biofilm.” This being the case, as the bacteria would be relatively inaccessible to both stain uptake and antigenic immunologic response.

The typing of the *P. acnes* into four groups, appreciating that type IA, and possibly IB, represent potential skin flora, validates that the remaining cases were not contaminants. Realizing that these 20 cases were representative of the total study, at least 70% of the positive *P. acnes* cultures were probably not skin contaminants. This being as strong of a confirmation as possible, combining the data synthesized through the technology available at the time of this initial study, and the insights provided since, that these anaerobic bacteria were residing in the intervertebral discs themselves.

In reviewing the rate of positive cultures according to individual categories (Table 3), it is noteworthy that in categories I and II, the highest overall bacteria presence rates were noted (63% and 65%, respectively), followed in order by the remaining three (III = 48%, IV = 30%, and V = 27%). It is possible that a herniated disc represents the more extreme pathological involvement of a single disc level, secondary to these organisms. Category III is typically a “pain only” condition, having no instability or neurologic involvement, and requiring complete disc excision (and interbody fusion) in order to fully relieve the pain with our present technology. The idiopathic deformity group (IV), which is currently felt to be a genetically driven condition, may represent “discs at risk,” in which deformity and pain may be triggered by the virulence of a microbial presence.<sup>29</sup> The low culture-positive rate seen in categories IV and V (controls) could represent a baseline intervertebral disc microbiome bacterial presence.

Several recent studies have shown what appears to be bacterial colonization of human tissues internal to the body surface. The growing body of evidence suggests that bacterial contamination cannot explain all of the reported cases.<sup>30</sup> Fritzell *et al.*<sup>31</sup> showed in a 10-patient study with painful discs, but without inflammatory change or infection, that 16S rRNA gene PCR (polymerase chain reaction) showed evidence of bacterial presence in two (20%) cases. A “culture only” study was

performed involving 54 posterior lumbar disc surgery patients that included culturing of the ligamentum flavum, muscle, and ambient air specimens; contamination was felt to be the cause of the low positive growth rate (22.2% total, 7.4% disc).<sup>32</sup> A Mayo Clinic study of nine male patients with nonimplant-associated *P. acnes* vertebral osteomyelitis documented the often atypical presentation of these patients; two with unremarkable imaging, five of nine with normal erythrocyte sedimentation rates, and three of nine with no evidence of infection on histopathology.<sup>33</sup> Uckay *et al.*,<sup>34</sup> in their review of 29 patients with painful *P. acnes* spondylodiscitis (28 with prior spinal surgery), found that fever at presentation was rare, as were positive blood cultures (1/15). Cultures were obtained on the excised discs from 83 patients who had lumbar disc replacement surgery in a study by Arndt *et al.*<sup>35</sup> Forty discs showed positive cultures, with *P. acnes* (18) and CN *Staphylococcus* (16) being the predominant microbe; no correlation with Modic changes was noted.<sup>35</sup> In contrast to those findings, Albert *et al.*,<sup>36</sup> in a double-blinded, chronic low back pain study involving 162 postdisc herniation (surgically and nonsurgically treated) patients, all of whom had Modic type I changes, found statistically significant pain resolution occurred in those undergoing a 100-day course of oral antibiotics *versus* placebo. A systematic review was performed in 2015 by Urquhart *et al.*<sup>3</sup> of 11 published studies on this topic. They found: “moderate evidence to indicate low virulent bacteria have a role in low back pain with disc herniation and moderate evidence for a relationship between bacteria and Modic Type I change associated with disc herniation.”<sup>3,37,38</sup> Li *et al.*<sup>39</sup> attempted to address these issues with a combined human/rabbit study. The human component showed growth in only three of 30 discs (cultured for 10 days), none *P. acnes*; the rabbits verified that “*P. acnes* could infect the degenerated lumbar discs by way of bacteremia.”<sup>39</sup> Finally, a recent editorial addressing the present understanding of spondyloarthritis and gut microbiota noted that evaluating, and trying to modulate gut microbiota is a very complex undertaking; however, various rheumatologic conditions may be associated with distinct bacterial profiles.<sup>40</sup>

In summary, the “biofilm within a biofilm” concept may contribute to the seemingly “sterile status” understanding of



the human spine intervertebral disc in the past. This study, with an overall positive disc culture rate of 45%, 45% of which were positive for *P. acnes* and 40% for *Staphylococcus*, lends strong support for an underlying, endemic presence of bacteria in the discs, which may contribute to pathologic conditions in some cases. Additional research is warranted to further validate these findings and clarify the physiology involved.

## ➤ Key Points

- ❑ An association of microbial colonization with progressive disc degeneration has been described.
- ❑ We found positive bacterial cultures in 76 of 169 discs (45%) removed at disc surgery.
- ❑ We report a significant association of disc herniation with bacterial outgrowth ( $P = 0.000088$ ).
- ❑ Bacterial biofilms, originating from the organism and/or the disc environment itself, may contribute to disc herniation and discogenic pain.

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